The use of some integrated control agents for powdery mildew in cucumber plants and their role in inducing systemic controlling

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Abstract :

This study aimed to evaluate the efficiency of the living fungi (Tv) Trichoderma viride and (Pc) Penicillium commune, the bacteria (Pf) Pesudomonas fluorescens pf.DS and the fungicide Dazim and their interactions in controlling powdery mildew disease in cucumber plants, induction of systemic resistance and identification of control agent .The results of the pathogenicity test of the powdery mildew fungus obtained from infected cucumber plants in greenhouses showed a pathogenic potential, where the percentage of infection severity was 45.02%. The results of the antagonism between the bio control fungi T. viride and P. commune also showed an equal antagonistic ability among them, as the degree of antagonism reached (3) according to the Bell scale.While the results showed the inhibition of P. fluorescens pf.DS for the living fungi T. viride and P. commune, which amounted to 56.11 and 55%, respectively. The results showed the effect of bio agents and the fungicide Dazim and their interactions, as the severity of infection decreased in the treatment of the Dazim, reaching 17.48%. It was followed by the treatments Tv + Pc + Pf, Pc + Pf, Tv + Pf, Tv + Pc, Pf, Pc and Tv, which ranged between 22.43 and 27.38% compared to the control treatment of 70.64%. The results of the study showed that the rate of peroxidase enzyme activity increased significantly in cucumber plants treated with powdery mildew, where the treatments Dazim and Tv+Pc+Pf gave the highest enzyme activity, which amounted to 7.100 and 6,610 units/g fresh weight, respectively, compared to the control treatment, which amounted to 0.802. unit/g fresh weight. The results showed the effect of different treatments in inducing polyphenol oxidase enzyme in cucumber plants treated with powdery mildew. The results showed the effect of different treatments on increasing the percentage of phosphorous element in the content of leaves of cucumber plants treated with powdery mildew, as the fungicide treatments Dazim and Tv + Pc + Pf gave the highest percentage, reaching 0.592 and 0.528%, respectively, compared to the control treatment of 0.248%.The results also showed an effect on the percentage of potassium in the content of the leaves of cucumber plants treated with powdery mildew, where it reached the highest in the treatments Dazim, Tv + Pc + Pf, Tv + Pf, Pc + Pf and Tv + Pc, reaching 5.21, 4.98, 4.19 and 4. 17 and 4.11%, respectively, compared to the control treatment of 1.18%.The results of the study also indicated that all treatments achieved a significant increase in the total chlorophyll content of the leaves of cucumber plants treated with powdery mildew in the greenhouse. were 11,250, 10,465, 9.648, 9.618, and 9.471 mg/100 g^{-1}, respectively, Compared to the control treatment of 4.800 mg/100 g^{-1}. The results showed that all the treatments used increased the productivity of one plant in the cucumber plants treated with powdery mildew .The treatments of Dazim and Tv + Pc + Pf achieved the highest weight, which reached 716.0 and 666.0 g / plant, respectively, compared with the control treatment of 116.0 g / plant.

Introduction :

The cultivated area is estimated at the option crop in Iraq, 6,9502 dunums, and with its productivity, amounting to 149302 tons for the year 2019, and in Basrah province, 1297 dunums, with its productivity, amounting to 4302 tons for the year 2020 [6].The cucumber plant is infected with many pests, including viral, bacterial, nematodes and pests, in addition to fungal diseases, including milky white disease. This disease spreads in many countries of the world and causes SPHEAROCA FULIGINEA and Erysiphe Cichoraarum mushroom In the greenhouses in Basrah province, the appropriate conditions

for the disease led to its spread, and they appear on the leaves, legs and fruits in the form of small spots that fuse and cover the parts with a white powder that gives a dild appearance of infection,The first signs of the infection of the milky white disease begin on the cucumber plant as white colors circular on the superficial leaves [57] and when the injury is advanced, the fungal colonies or stains are united and expanded quickly and cover the surfaces of the axial leaves completely under the appropriate environmental conditions [63].Chemical fungicide s were used to control the disease. In 2012, two fungicides were allowed to control the diligent white disease in the cucumber plant in Sweden, which are Amistar. Effective to it penetrates the tissue of the plant and prevents the infection and disrupts it, but its effectiveness is low. Where it was used for a long time, which led to the development of the characteristic of resistance in the fungi of the milky whiteness against it. "Fungazil is a fungicide that has a repressive effect on the nurses and allows its use twice a year while the first fungicide is three times for each cultivation season [45] and [39]. indicated the use of fungal fungicide s early to prevent fruitful whiteness on cucumber, because most fungal fungicide s used in control are preventive fungicide s, and there are some recorded fungicides used to control the milky white disease in the cucumber plant, including systemic functions and myclobutanil (Nova®), azoxystrobin (Quadris®), Trifloxysroin (FLINT®) and phytical philanthropies such as Chlorothhalonil, which are multi-site inhibitors, therefore there is a lower possibility of developing the status of resistance by nurses [51]. Bio-agent were also used to combat the disease, which was between [38] and [33] in a study on the cucumber plant with mild whiteness when using T.Harzianum as a vital preparation to combat the disease as it led to an increase in production after a reduction in the severity of the infection and the number of universities in addition to its being A environmentally friendly, and the bio fungi can be used by T.Harzianum. also used a group of bio agent, including B. Subtilis, P. Fluorescens, and T. Harzianum mushrooms, where it led to a decrease in infection to levels without economic embarrassment. [14] it explained that this fungi is characterized by its possession of several mechanisms such as contrast, competition and intrusion towards A.alternata [54] and [56].The results of the efficiency of biological fungi are evaluated in inhibiting the growth of albicia, on the cucumber plants, at a concentration of 50 %, and it was a penicillium spike, as the inhibition rate was 8.71 % [1]In view of the importance and spread of this disease on the cucumber plant in some areas of its cultivation in Iraq in general and in Basrah province in particular, and the lack of specialized studies on this disease, so the need for such a study aimed at studying the disease and the possibility of its integrated control and the urges of systemic resistance in plants The option against the nurse.

Materials and methods:

Diagnosing the cause of powdery mildew on cucumber plants

Samples were brought from the areas of (Al-Zubair, Shatt Al-Arab and Al-Madina) to the laboratory for the purpose of diagnosing the pathogen, both morphologically and microscopically. The pieces of plant tissue were examined directly using a light microscope. The method [22] was also followed in preparing the samples for the purpose of microscopic testes, where the tablets taken from the affected leaves with a diameter of 1 cm with a cork perforator are usually placed in a vial of 10 ml containing 5 ml of a solution consisting of ethyl alcohol (100%) and acetic acid in a ratio of 1: 3 which has been prepared in advance. It was left for several hours until the chlorophyll was removed and the color of the tablets became transparent, then it was washed quietly with distilled water, then the tablets were kept in a 3 ml Lacto phenol solution for 8 hours at a temperature of 60 ° C. Then the discs were fixed on slides with a drop of Trepan blue 5% pigment placed on them for a period of 30-15 minutes, then the slide cover was placed on

them, taking into account that no air spaces were left, then the samples would be ready for tests under the microscope.

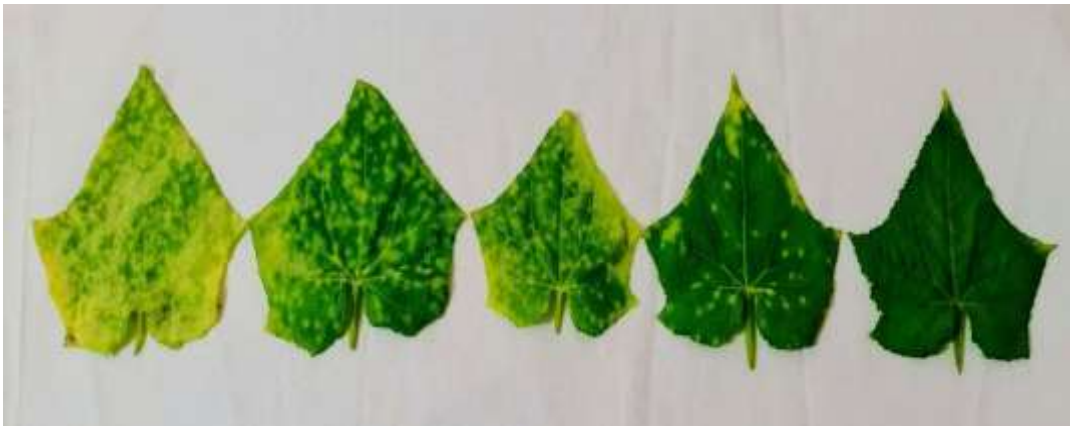
Pathogenicity test in the greenhouse

The pathogenicity of the powdery mildew fungus was tested in 5 kg plastic pots containing a mixture of soil and peat moss in a ratio (1: 3) sterilized with commercial formalin prepared from a solution consisting of formalin: water (50: 1), which was used at a ratio of 3 liters / m3 and left the soil mixture. for 3 days before use [4], Then the seedlings of cucumber plants grown in cork ponds, Wesam F1 cultivar, produced by Volo Agriseeds of American origin, were transferred to it. After reaching the fourth true leaf, the cucumber plants were inoculated with the powdery mildew fungus.The process of artificial insemination was conducted according to the method [13] using a tower made of cardboard, 90 cm high and 70 cm in diameter, by placing the pots in which cucumber seedlings were planted at the base of the pollination tower and spraying with water. The spores were spread on the surfaces of the plants by quietly moving the leaves of the infected cucumber plants on them, then leaving the inoculated plants for 5 minutes before raising the tower.

details	Degree
No infection	0
From 15 - 1% of the leaf area covered with powdery mildew	1
30-16% of the leaf area is covered with powdery mildew	2
From 60 - 31% of the leaf area covered with powdery mildew	3
100-61% of the leaf area is covered with powdery mildew	4

The McKinney equation presented in [11] has been applied as follows:

infection severity %=$\frac{degree\ x\ number\ of\ infected\ leaves\ of\ each\ degree\ (total)}{highest\ degree\ x\ total\ number\ of\ leaves}$×100



Picture (1) Pathological evidence of powdery mildew in cucumbers

Preparation of the vaccine and the occurrence of infection:

fungi inoculum was prepared from pre-prepared infected plants and the previous method [13] was followed for the inoculation of plants. the culture medium used in the development of bio agent:

Several culture mediums have been used to develop, purify and preserve isolates of the biological fungi T. viride and P. commune, including Potato Dextrose Agar (PDA) and P.D (PDB) medium. Broth As for the bacteria P. fluorescens, it was used to grow and activate it with a median of (KB) King Broth and (KBA) King B. Agar.

Bio control agents:

The bio agents T.viride and P.commune and P. fluorescens pf.DS were obtained from Prof. Dr. Diaa Salem Al-Waeli, College of Agriculture, University of Basra. The fungi were activated by taking fresh growths from the edges of the colony of fungi using a sterile needle. The fresh growths were transferred to Petri dishes containing sterilized plant media (PDA), separately, and then the dishes were incubated at a temperature of (±2). 25 ºC) for a week,Then part of the colony was transferred to sterile plastic tubes with a capacity of (10 ml) containing sterile slanted culture medium (PDA), then incubated at a temperature of (2 ± 25 ° C) for a week and then kept in the refrigerator until use with the renewal of the isolates in those tubes whenever called for. the need for that. The bacterial inoculum was prepared using a liquid (KB) medium, and sterilized the medium with an autoclave device at a temperature of (121 °C) and atmospheric pressure (1.5 kg/cm2) for 20 minutes, after which the medium was left to cool and then distributed on sterile flasks volume (250 ml) and (100 ml / A beaker) and the bacterial inoculum was added to it in an amount of (1 ml) for each beaker, after which it was incubated at a temperature of (2 ± 28 ° C) for (48) hours [47].

Propagation of the bio control fungicide vaccine:

The inoculation of T. viride and P. commune, both separately, was prepared using the seeds of local millet Panicum miliaceum L. after being thoroughly washed with water to remove dirt and impurities from them; They were soaked in water for (6) hours and left on a piece of gauze for half an hour to remove the excess water and dried at laboratory temperature. Then they were placed in glass beakers with a capacity of (250 ml) at a rate of (100 g / beaker). Then a little water is added to it to moisten it, then sterilize the beakers with a sterilizer at a temperature of (121 °C) and atmospheric pressure (1.5 kg / 2 cm) for an hour, Then the flasks were left to cool and each flask was inoculated with (10) tablets with a diameter of (0.5 cm) from the colony of living fungi growing on PDA medium at the age of (7) days, both separately. days, taking into account the shaking of the flasks every (3-2) days to ensure the distribution of bio-fungi on all seeds [29].

Growth velocity test between T. viride and P.commune

In this test, the double culture method was used to test the ability of biological fungi to antagonize with each other.The petri dish containing the sterile PDA was divided into two parts. The centre of the first section was inoculated with a drop disc (0.5 cm) from the colony of the bio fungi P.commune at the age of (7) days, and the centre of the second part was inoculated with a drop disc (0.5 cm) from the fungi colony. The bio T. viride at the age of (7) days and with three replicates. For control, a Petri dish containing the prepared and sterile PDA inoculated with a dropped disc (0.5 cm) from the colony of the bio fungus P. commune and another Petri dish containing the prepared and sterilized (PDA) medium was used. Inoculated with a drop disc (0.5 cm) from the colony of the biological fungus T. viride, then the plates were incubated under temperature (2±25 °C) when the fungal growth in the control treatment reached the edge of the plate, and the degree of antagonism between them was calculated according to the [19] scale.

P. fluorescens pf.DS inhibition test for T. viride and P.commune

The Spotting method was used to test the antagonistic efficacy of P. fluorescens pf.DS bacteria against the live fungi, both individually. Petri dishes with a diameter of (9 cm) containing the prepared and sterilized (PDA) medium were inoculated with the inoculation of bacteria growing in (KB) medium of age (KB) (48) hours by (4 drops/dish) by (0.1 ml/drop) on the edges of two perpendicular diameters and at a distance of (1 cm) from the edge of the dish. Then the dishes were incubated at a temperature of (25 °C) for (48) hours [61]. Then the center of each dish was inoculated with a drop disc (0.5 cm) taken from the edge of the T. viride and P.commune cultures grown on PDA medium at the age of (7) days with three replicates, leaving a control treatment without bacteria.Then the dishes were incubated at a temperature of (25 °C), then the percentage of inhibition was calculated after the growth in the control treatment reached the edge of the dish according to the About equation contained in [11]:

$$\text{inhibition\%} = \frac{\text{The average diameter of the fungi colony in the treatment} - \text{the average diameter of the fungi colony in control}}{\text{The average diameter of the fungi colony in control}} \times 100$$

The fungicide used in the study:

Dazim 50sc is a systemic fungicide in the form of an emulsion from the Carbendazim 50%sc group, fast-acting and works in two directions from bottom to top and from top to bottom. It eliminates a wide range of fungi that attack the shoot system, including powdery mildew fungi. It also eliminates fungi that affect the root system and is used as a fungicide . Preventive and curative. Produced by the Chinese company King Quenson and uses a spray on the leaves at an amount (125 - 75 ml / 100 liters of water)

Studying the effect of different treatments on powdery mildew on cucumber plants in greenhouse conditions:

A mixture of soil and peat moss was prepared in a ratio of (1:3). Soil: pre-sterilized peat moss was placed in plastic pots with a capacity of (5 kg) soil, and the previously prepared fungal pollen was added to the millet seeds of the biological fungi T. viride and P. commune at a ratio of (5 g / kg). Soil) for each mushroom was mixed well with the soil and watered the pots daily for a period of (3) days on the other hand, the seeds of the cucumber plant Wissam F1 were planted in cork tubs containing sterilized peat moss, and after the appearance of the true leaves, they were transferred to plastic pots. The bacteria P. fluorescens pf.DS was added at a percentage (5 ml / seedling) and at a concentration of 10-7 CFU / ml to the soil around the root zone of the seedlings.As for the Dazim fungicide, it was sprayed on the leaves with a concentration of (1 ml / liter) of wetted water before infection with the powdery mildew fungus for a period of (3) days. Control of powdery mildew inoculum obtained from infected cucumber plants (a source of infection)The process of artificial insemination was conducted according to the [13] method, and after (5-4) days, symptoms of powdery mildew infection began to appear on the leaves of the plants, and service operations were carried out for the plants, including watering and fertilizing, where the compound fertilizer N-P-K (20-20-20) was added to the plants. The production of the Modern United Company of Jordan at a rate of (3 g/us) after two weeks of planting and the fertilization process was repeated two weeks after the first fertilization process, according to the requirements of the crop and whenever needed.

The experiment included applying the following treatments:

1- Treatment of T. viride

2- Treatment of the fungus P. communine

3- Treatment of P. fluorescens pf.DS . bacteria

4- T. viride + P.commune . treatment

5- Treatment of T. viride + P. fluorescens pf.DS

6- Treatment of P.commune + P. fluorescens pf.DS

7- Treatment of P.commune + P. fluorescens pf.DS + T. viride

8- Treating the fungicide

9- Control treatment

The severity of infection of cucumber plants infected with powdery mildew was calculated after (7) days of inoculation with the pathogenic fungus, using the previous pathological evidence and the previous equation.

Determination of the total chlorophyll content of leaves (mg. 100 g^{-1}):

The total chlorophyll was estimated in the leaves of cucumber plants, where the leaves were taken from fresh plants (the fourth leaf) by taking a weight of (0.5 g) from the leaf, which was crushed by a ceramic mortar with the addition of (10 ml) of acetone diluted 80% [36] for the purpose of extracting chlorophyll. Then it was filtered by means of filter paper to isolate the dye solution and then it was kept in tubes and placed in a centrifuge (center fuge) for (10) minutes and for a number of (6000) cycles. Then the stencil was taken and placed in a spectrophotometer to measure the optical absorption of the dyes at the wavelengths (645) for chlorophyll B and (663) for chlorophyll A, then the amount of chlorophyll was estimated (mg / L) according to what was stated in [64]

According to the following equation:

Total chlorophyll (mg/L) = 20.2 x O.D (645) + 8.02 x O.D (663)

Note that O.D represents the absorptive reading of the device.

In order to convert the amount of chlorophyll from (mg/L) to (mg/100g) the weight of the sample, we apply the equation assumed by [78]. It is as follows :

$$\text{mg/100 g} = \frac{100}{Weight\ of\ the\ measured\ sample\ in\ grams} \times \frac{mg/L}{1000\ mL}$$

Determination of peroxidase enzyme content in leaves:

Samples were taken from the leaves of cucumber plants and placed in bags of polyethylene, written on each bag of the type of treatment, and placed in a box containing ice and transferred to the laboratory; (150 mg) fresh weight was taken from the leaves and washed with distilled water free of ions, then the sample was mashed well with a ceramic mortar and (2.5 ml) of the buffer solution was added to it Potassium phosphate buffer at a concentration of (0.05) molar, which consists of potassium mono-hydrogen phosphate (K2HPO4) and phosphate Potassium is dihydrogen (KH2PO4) and pH (6).Al-Dari's phosphate solution was prepared by taking (13.2 ml) of (K2HPO4) one molar + (86.8 ml) of (KH2PO4) one molar, then taking (50 ml) of the mixture and completing the volume to (100 ml) of distilled water to obtain Potassium phosphate buffer at a concentration (0.05) molarity and PH equals (6), then put the mixture "leaves + Al-Dari's phosphate solution" in a centrifuge at (1200) cycles / minute for a period of (20) minutes;The filtrate was taken and (250) microliters of each of Gauiacol dye at a concentration of (0.5%) and hydrogen peroxide at a concentration of (0.3%) volume/volume and (2.5 ml) of the buffer were added to it.After that, the amount of absorption was read in a spectrophotometer with a wavelength of (470) nm [46].The enzymatic activity was estimated on the basis of the absorption unit / g of fresh weight according to the following equation:

$$\textbf{\textit{Enzymatic activity (uptake unit / g fresh weight of leaves)}} = \frac{reading\ device}{(Form\ weight\ /\ Extract\ size) * The\ volume\ taken\ for\ reading}$$

Determination of the content of polyphenol oxidase enzyme in leaves:

A - Extraction

The method was followed [43] to obtain the crude enzymatic extract, where leaves from cucumber plants were taken and placed in polyethylene bags marked, each according to its treatment, and placed in a cork box containing ice and transferred to the laboratory, then (2 g) of leaves were taken.

It was placed in an electric mixer (Blender) with a volume of 1.5 liters and added to it (50 ml) of cold Darry potassium phosphate (0.2 M) and pH (5.7), which was prepared by dissolving (1.30 g) of KH2PO4 in distilled water and dissolving (0.066 g) of K2HPO4 in Distilled water and mix the two solutions and add to them (0.88 g) ascorbic acid (5 mm) molar with (5 g) of PVP (Poly Vinyl Pyrolidon).Where the pH function was modified to (5.7) and the volume was completed to a liter of distilled water, the homogenization process was conducted until a homogeneous suspension was obtained. Filter the mixture through a cheese cloth, followed by centrifugation at a speed of (5000) gx for (10) minutes in a refrigerated apparatus; Separate the clear solution (the crude enzymatic extract) and store it in the refrigerator at a temperature of (4°C) before carrying out the enzyme precipitation process.

B- Determination of polyphenol oxidase activity by quantitative method

The method mentioned by [71] was followed in the estimation, by following up on the increase in the light absorption (Absorbance) at a wavelength of (420) nanometers using a spectrophotometer, which resulted from the oxidation of catechol as a substrate for the enzyme.

C - Assay Procedure

The reaction mixture was prepared by mixing (1 ml) of Dart's solution with (1 ml) of a substrate solution at a concentration of (20 mmol). This solution was prepared by dissolving (0.248 g) of catechol in a solution of potassium phosphate and completing the volume to (100 ml). At a temperature of (20 °C) (1 ml) of the enzymatic solution was added with rapid mixing;This solution was quickly used to zero the previous spectrophotometer by adjusting it to a wavelength of (420) nanometers. After that, the change in light absorption was recorded during (5) minutes of reaction at a temperature of (30 °C), then the readings were recorded after taking three replicates for each reading.

The number of enzyme units per ml of enzyme solution was calculated from the following equation:

$$\text{number of enzyme units/ml} = \frac{(dilution\ x\ change\ in\ light\ absorption\ in\ 5\ minutes)}{(0.001\ x\ time)} \times 100$$

The enzymatic unit of polyphenol oxidase is defined as the amount of enzyme that causes an increase in light absorption of (0.001) per minute at a wavelength of (420) nanometers.

Methods for estimating the content of phosphorous and potassium in cucumber leaves:

The method was followed [25] where (2.0 g) of samples were taken and placed in special glass flasks for digestion, then (5 ml) concentrated sulfuric acid H2SO4 (98%) was then added to it. It was placed on a thermostat at a temperature of (120 °C) for half an hour, after which the samples were cooled and (3 ml) of the acidic mixture was added to it, which consisted of concentrated sulfuric acid and concentrated perchloric acid H2SO4 + HClO4 at a concentration of (96 + 4%), then the temperature was raised to (350 °C) to obtain a clear, transparent solution, Then the solution was cooled and transferred volumetrically to a volumetric flask with a capacity of (50 ml), then the volume was completed with distilled water to (50 ml), then the samples were available for the determination of mineral elements. The color intensity of the solution was measured with a

UV-1700 spectrophotometer at a wavelength of 700 Nm. As for potassium, potassium was measured in the digestion solution after diluting it with distilled water using a flame photometer, according to the method described in [60].

statistical analysis :

The Completely Randomized Design (C.R.D) was used in the laboratory experiments, while the experiments were coundected inside the greenhouse according to the Randomized Completely Block Design (R.C.B.D) and all the averages were compared according to the least significant difference (L.S.D) test method at the 0.01 level in Laboratory experiments and 0.05 for experiments inside the greenhouse [3]. The statistical program Genstat discovery edition was used.

Results and discussion :

Diagnosing the cause of powdery mildew on cucumber plants:

The symptoms and signs of powdery mildew disease on the leaves samples of the infected cucumber plant were characterized by the form of white spots on the surface of the leaves, which are carriers and spores of the causative fungus. These spots appear on the stems, and when the infection progresses, these colonies or spots unite and cover the surface of the leaves, and then these spots turn brown, and this leads to the drying of the leaves and their death, but the leaves remain hanging on their necks. Their small size and low production may lead to plant weakness and death [53]and between the microscopic examination of thin pieces of infected plant tissue, where the mycelium is branched and colorless, divided by transverse walls, the conidia are tall to the top and short, and the conidia are carried in chains on the conidia, and they are unicellular, colorless and oval in shape at the end of the season, the sexual phase of the powdery mildew fungus did not form on the plant parts, which is in the form of dark-walled closed spherical cyst fruits and simple mycelial appendages containing one cyst in the case of Sphaerotheca fuligenea and several cysts in Erysiphe cichoracearum and each bag contains a number of cystic spores according to for taxonomic keys [62].It was also found in another study when plants of the cucurbit family (cucumber and squash) were infected with the pathogens Sphaerotheca fuligenea and the fungus Erysiphe cichoracearum, where the two fungi were seen together on the two plants. Finally [5], so we believe that the cause of powdery mildew disease on cucumber plants is either S. fuligenea or E.cichoracearum .

Pathogenicity test of powdery mildew fungus on cucumber plants:

The results of the pathogenicity test (Figure 1) showed that the powdery mildew fungus obtained from infected cucumber plants inside greenhouses had a pathogenic ability to infect cucumber plants, where the percentage of infection severity was 45.02%The infection leads to yellowing, dryness and death of leaves, secretion of the pathogen with enzymes that break down cell walls, weakness of the host plant's defense system, weak ability to produce inhibitors and enzymes for the pathogen, as well as its possession of some important nutrients for the pathogen [67]. The plant is affected physiologically, as the transpiration process increases, especially at night, and also the injury leads to an increase in respiration and thus leads to a decrease in the process of photosynthesis [37].

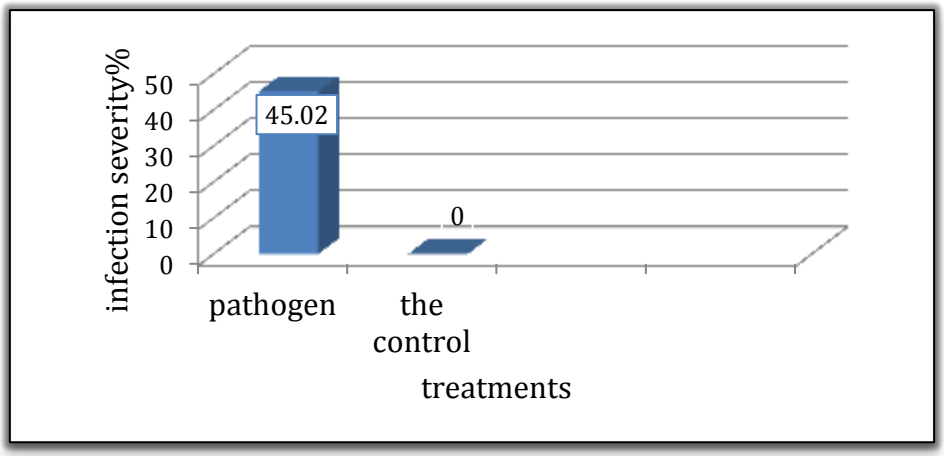
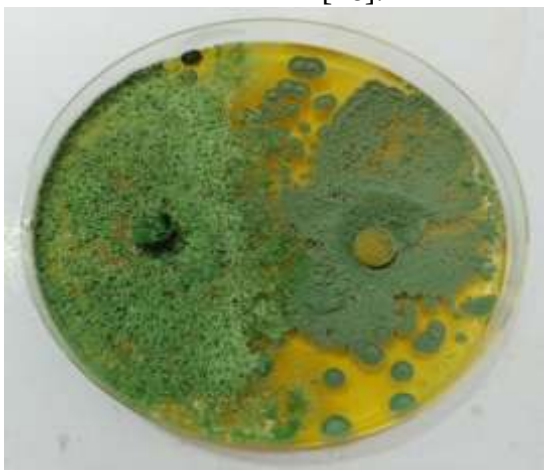


Figure (1) The percentage of infection severity of powdery mildew on cucumber plants

Growth assay between T. viride and P. commune

The results of the antagonism experiment between biocontrol fungi (Picture 2) indicate that these fungi have equal antagonistic ability between them on the PDA culture medium according to the [19] scale. The degree of opposition between them reached (3). This degree explains the efficiency of these two fungi in their opposing ability between them, as each covers half of the dish. The growth rate of the colony diameter for each of them was (4.5 cm) compared to the growth of fungi for a unit on the culture medium, which reached (9) cm for both fungi and each separately The reason for the antagonistic efficiency of these fungi is due to the different mechanisms they possess, as T. viride has the ability to compete for space or for nutrients, and the production of volatile metabolic substances that are unique to this type and the production of antibiotics [9]. This fungus also possesses the ability to produce cell wall degrading enzymes (cellulose and hemicellulose), which work within the mechanisms of antagonism and parasitism to destroy the cell wall, such as the enzyme Protease, Chitinase, Cellulase and B-1,3glycanase [41] and [28].

Penicillium spp. has a high antagonistic ability due to its production of enzymes such as B-1,3glycanase and cell wall-degrading chitinase. This fungus also stimulates the systemic resistance of plants against pathogens [26].



Picture (2) Measurement of growth between T. viride and P.commune

P. fluorescens pf bacteria inhibition test. Ds of the living fungi T. viride and P.commune

The results of this experiment (Figure 2 and Figure 3) showed that the bacteria P. fluorescens pf. The ability of DS to inhibit the growth of T. viride in the PDA medium , as the percentage of inhibition was 56.11%, and the fungus had no effect on the growth and density of bacteria.This result is consistent with the findings of [8] which showed that P. fluorescens had the ability to inhibit the bio fungus T. viride, where the percentage of inhibition was 55.4%. While the percentage of bio-inhibiting P.commune was 55%, and the bio-fungus had no effect on the growth and density of bacteria.The reason may be due to the excelled of the mechanisms by which these bacteria operate over the mechanisms by which the bio fungi operate, or the reason may be due to the rapid spread and growth of bacteria in the medium.

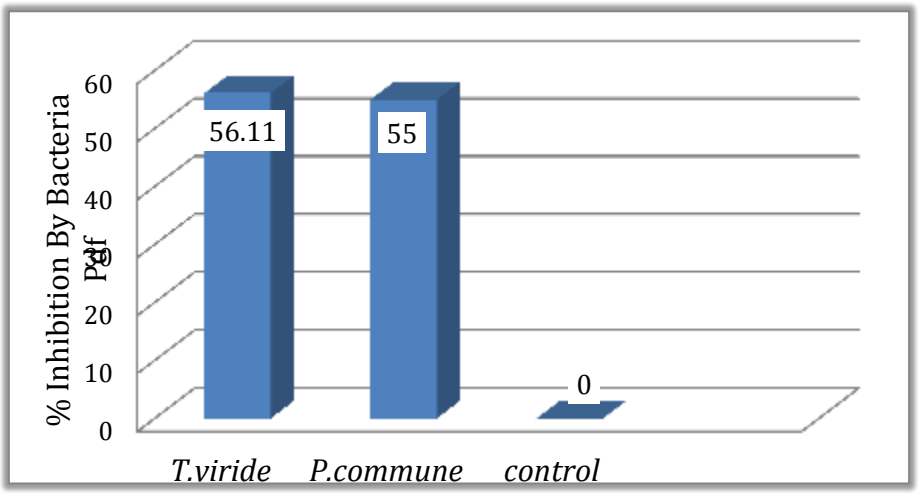


Figure (2) Inhibition of P. fluorescens by T. viride and P.commune in PDA culture meduim

Picture (3) The effect of P. fluorescens on the growth of T. viride A- and P.commune B- in PDA culture medium

The effect of different treatments on the severity of powdery mildew disease on cucumber plants in greenhouse conditions:

The results showed (Table 1) that the treatment of the Dazim had the greatest effect in reducing the severity of infection, reaching 17.48%, compared to the control treatment, which amounted to 70.64%, followed by the interaction treatment of bio control agent Tv+Pc+Pf, where the rate of infection severity was 22.43%.The results indicated that there were no significant differences between the interaction treatments Tv + Pc, Tv + Pf and Pc + Pf, as the rate of infection severity of the pathogenic fungus was 24.48, 24.56 and 24.98%, respectively. As for the single treatments of bio control agent, where the Pf bacteria treatment achieved the lowest rate of infection severity, which reached 25.39%, followed by the treatment of the bio fungus Tv with a severity of infection of 27.38%, and then the treatment of the bio fungus Pc with a severe infection rate of 28.07% compared to the comparison treatment of 70.64%. These

results are consistent with [59] who showed the ability of P. fluorescens bacteria to reduce the percentage of infection severity with the pathogenic fungus F.oxysporum f.sp.melonis, and the efficiency of this bacteria in reducing the rate of infection severity may be due to its ability to secrete antibiotics such as Phenzain-1 -caroxylate [74]or it may be due to the secretion of this protease enzyme, which is anti-pathogenic to many pathogens, and its production of antibiotics such as Pyoluteorin and 2,4-diacetylphloroglu cinol, which is a key factor to reduce the production of fusaric acid produced by the pathogenic fungus F.oxysporum in addition to the production of hydrogen cyanide [31].[23] indicated the efficiency of P. fluorescens bacteria in reducing the percentage of infection severity to its ability to suppress pathogenic fungi through its production of iron chelates as well as its production of antibiotics, its competition for space and food, and its induction of systemic resistance. This study was consistent with what [32] mentioned the role of the bio-fungus Trichoderma spp. In reducing the severity of the infection, the reason for this is due to the effect of the bio-agent on the pathogen and the reduction of its pathogenicity as a result of its direct parasitism and competition for space and food, as well as the production of antibiotics and enzymes. It was also in agreement with what was mentioned by [73] who indicated the ability of T.viride to reduce the severity of infection with pathogenic fungi through the secretion of the extracellular protease enzyme, which has a role in this, [21] the effective effect of Swollenin protein that was isolated from some types of fungi. The biogenic fungus Trichoderma spp. against some pathogens He [12] pointed to the importance of bio control using the bio fungi T.viride and T.harzianum, as they achieved an average reduction in the treatment of powdery mildew and downy mildew on watermelon, cucumber and pepper alone or when mixed together. As for the bio-fungus Penicillium sp. The positive effect in reducing the severity of infection is due to its ability to secrete many antibiotics that destroy the walls of pathogenic fungi cells and competition for food and place, in addition to its ability to parasitize the pathogenic fungus [54] and [56],The reason for this reduction in the severity of the infection with the pathogenic fungus may be due to the ability of the bio fungus Penicillium sp. In providing protection for plants as a result of its colonization of plant roots and its competition for space and food, in addition to its ability to produce gibberellins [76].This result was in agreement with [42] who showed the role of the bio fungus P. chrysogenum in the production of pathogenic antifungal proteins as it secretes small cationic proteins that are rich in cysteine with anti-pathogenic action. It also has the ability to induce systemic resistance in plants against pathogens, as well as the production of chitinase enzymes and glucanase B1-4 glucanase enzymes [27].

Table (1) The effect of different treatments on the severity of powdery mildew disease on cucumber plants in greenhouse conditions

% infection severity	treatments
70.64	Control
17.48	Dazim
27.38	*Tv*
28.07	*Pc*
25.39	*Pf*

24.48	*Tv+Pc*
24.56	*Tv+Pf*
24.98	*Pc+Pf*
22.43	*Tv+Pc+Pf*
5.09	L.S.D 0.05

* Each number in the table represents an average of three replicates

The effect of different treatments on the concentration of peroxidase and polyphenol oxidase enzymes in leaves of cucumber plants treated with powdery mildew in greenhouse conditions:

The results of (Table 2) showed that the rate of peroxidase enzyme activity increased significantly in cucumber plants for all treatments used in the study compared with the control treatment (Control). Biological control Tv+Pc+Pf with a value of 6.610 units/gm wet weight compared to the control treatment which amounted to 0.802 units/gm wet weight.While the enzymatic activity was 6.200, 6.030 and 5.400 units/gm wet weight for the interaction treatments of other bio control factors Pc + Pf, Tv + Pf and Tv + Pc, respectively. As for the single treatments of biological control agents. As the treatment of the bacterial bio control Pf achieved an increase in the rate of enzymatic activity over the rest of the other single treatments, which amounted to 4.210 units/gm wet weight, followed by the treatment of the fungal bio control Pc with an enzymatic activity that amounted to 3.200 units/gm wet weight, while the enzymatic activity of the treatment of the fungal bio control Tv 3.050 Unit/gm wet weight compared to the control treatment, in which the enzymatic activity was 0.802 units/gm wet weight.Several studies indicated the ability of the bacterial bio-resistant P. fluorescens to induce systemic resistance in plants against a wide range of pathogenic fungi as a result of increasing the production of peroxidase enzyme in it.[69] showed the efficiency of these bacteria in inhibiting the growth of pathogenic fungi to their ability to induce plants to secrete many enzymes, including Peroxidase, Phenylalanine ammonia-layase and Polyphenol oxidase, which have a role in stimulating plant growth. The reason for the positive effect of the antibiotic penicillium spp. To the important mechanisms of this bio fungus, such as the induction of systemic resistance (ISR), which is linked to the activation of some genes that produce pathogenicity-related proteins (P-RP) and the production of antioxidant enzymes such as peroxidase by plants [72]. Several studies showed the efficiency of the fungal bio-resistant Trichoderma spp. In inducing systemic resistance against a wide range of pathogens due to increased production of peroxidase enzyme in plants.It was reported [11] that the efficiency of the bio control of T. viride and T. harzianum in stimulating resistance in cowpea plant against the fungus Macrophomina phaseolina was by increasing the enzymes of peroxidase, polyphenol oxidase and cellulase. [50] pointed to the role of the biological fungus T. viride in increasing the activity of the enzyme Polyphenol oxidase, Peroxidase and Chitinase. As for the polyphenol oxidase enzyme, the results of the study (Table 2) indicate that there are significant differences between the treatments used in inducing the polyphenol oxidase enzyme in cucumber plants treated with powdery mildew compared to the control treatment (Control). The two treatments of the chemical fungicide and the interaction treatment of the bio control agents Tv+Pc+Pf achieved the highest enzymatic activity among the treatments used in the study, reaching 4.678 and 4.162 units/ml, respectively, compared to the control treatment in which the enzymatic activity reached 0.116 units/ml. While the other interaction coefficients of the

bio control agents Pc + Pf, Tv + Pc and Tv + Pf were similar in the enzymatic activity, which amounted to 3.820, 3.732 and 3.600 units / ml, respectively.As for the single treatments of the bio control factors Pf, Pc and Tv, the enzymatic activity reached 3.010, 2.961 and 2.828 units/ml, respectively, compared to the comparison treatment of 0.116 units/ml.This study was consistent with many studies that showed the ability of P. fluorescens bacteria to reduce the growth of pathogenic fungi that were not limited to control, as it is believed that they have the ability to stimulate enzymes such as peroxidase and polyphenol oxidase responsible for systemic resistance in plants [24] and [2].Several studies reported that the bio fungus Trichoderma spp. It has the ability to stimulate the secretion of the enzyme polyphenol oxidase and peroxidase, and the presence of these enzymes is associated with high resistance in the plant and raising the content of the concentration of phenolic substances as a result of infection with pathogens that are considered as a primary response through the oxidation of phenols, as the peroxidase enzyme works on the oxidation of phenol and the polyphenol oxidase enzyme works On the oxidation of catechol in addition to its role by participating in the synthesis of suberin and lignin after the polymerization of phenolic compounds in the places of injury [44] and [40].

Table (2) Effect of different treatments on the activity of the enzyme Peroxidase (unit/g) Polyphenol oxidase (unit/ml) in cucumber plants treated with powdery mildew fungus

enzymes		treatments
P.P. oxidase	Peroxidase	
0.116	0.802	Control
4.678	7.100	Dazim
2.828	3.050	*Tv*
2.961	3.200	*Pc*
3.010	4.210	*Pf*
3.732	5.400	*Tv+Pc*
3.600	6.030	*Tv+Pf*
3.820	6.200	*Pc+Pf*
4.162	6.610	*Tv+Pc+Pf*
0.415	0.669	L.S.D 0.05

* Each number in the table represents an average of three replicates

The effect of different treatments on the content of phosphorous and potassium in leaves of cucumber plants treated with powdery mildew in greenhouse conditions:

The results of the study (Table 3) indicate that the chemical fungicide and Tv+Pc+Pf treatments gave the highest percentage of phosphorous content in the leaves, which amounted to 0.592 and 0.528 percent, respectively, compared to the control treatment, in which the percentage of

phosphorous was 0.248 percent. As for the other interaction treatments for bio control agents, the percentage of phosphorous in it was 0.467, 0.450 and 0.433% for the treatments Pc+Pf, Tv+Pf and Tv+Pc, respectively. As for the single treatments of the bio control agent, the treatment of the bacterial bio-control Pf achieved the highest percentage of the leaves content of the phosphorous element, which amounted to 0.398%.It was followed by the treatment of the fungal bio control Pc with a phosphorus percentage of 0.376%, while the fungal bio control Tv treatment gave 0.364% of the phosphorous content of the leaves compared to the control treatment in which the percentage amounted to 0.248% of the phosphorous element.These results were consistent with many studies, where [18] indicated the efficiency of P. fluorescens bacteria in dissolving organic phosphorous and releasing organic acids, and thus phosphorus becomes more accessible to plants.Also, these bacteria fluorescens.P are characterized by their ability to stimulate plant growth, which is called the growth-activated root bacteria, where these bacteria have the ability to fix nitrogen and increase the readiness of the important elements in the soil solution and their impact on the shape and growth of roots and their ability to build or change the concentration of growth regulators and the ability to Reduce the effect of pathogens or antagonism through the formation of iron-chelating siderophores, antibiotics, cyanide and some enzymes such as chitinase . These bacteria can also build the enzyme ACCdeaminase, which in turn lowers the concentration of ethylene and thus stimulates growth. These bacteria encourage nutrient absorption and accelerate the start of resistance to stress and contribute to the dissolution of inorganic phosphate ions solubbilization in vitro p and the conversion of organic phosphate ions as well as the construction of enzyme B 1,3-glucanase [17] .He explained [75] the bio role of Penicillium spp. In recycling the phosphorous in the soil, turning it into a form ready for absorption by the plant, and then this contributes to increasing growth as it has the ability to produce antioxidants and antibiotics that discourage the growth of pathogenic fungi [77] and [52].This came in agreement with [68] who indicated the ability of the biological fungus T. viride to increase the elements of phosphorous, copper, potassium, zinc, nitrogen and manganese in the plant content as a result of increasing the availability of these elements and the ability of the bio fungi to compete with different soil organisms located in the root area.As for potassium, the results (Table 3) showed that the Dazim treatment was superior to the rest of the other treatments, as it amounted to 5.21% compared to the control treatment in which the percentage reached 1.18%, followed by the treatments Tv+Pc+Pf, Tv+Pf, Pc+Pf and Tv +Pc Where they amounted to 4.98, 4.19, 4.17 and 4.11 percent, respectively, which significantly excelled the control treatment, where the percentage reached 1.18 percent, as for the single treatments for the bio control agent Pc, Tv and Pf, where the percentage converged, which amounted to 3.66, 3.47 and 3.36 % respectively, compared to the control treatment of 1.18%.The use of bio control agents improved the availability of potassium and encouraged plants to absorb it. This was in agreement with [20] who indicated the ability of P. fluorescens bacteria to colonize plant roots and secrete saliciylic acid, where this acid stimulates systemic resistance in plants, and this in turn encourages Growth and uptake of nutrients by plants.This study was consistent with [65] who indicated the efficiency of the mechanisms possessed by the bio fungus Trichoderma spp. In increasing the facilitation of the mineral elements for plants, especially the element phosphorous, magnesium and potassium, which are not available for the plant, and the reason for the high percentage of potassium in plants treated with this biological fungus is due to its efficiency in increasing the surface area in the roots and to the role played by the hyphae of this bio control in the absorption of nutrients Including potassium, compared to untreated plants, in which the percentage of this element is low.[16] also referred to the efficiency of

the biological fungus Penicillium sp. In increasing the available -made nutrients such as zinc, potassium and iron, and this study was in agreement with what was found by [58] who indicated the efficiency of the biological fungus P. chrysogenum internal roots in stimulating the growth of plants and encouraging them in harsh ecosystems, because these bio agents play an environmental role that is not enough It destroys various nutrient sources and accelerates the process of nitrogen mineralization and improves the acquisition of nutrients, which in turn stimulates the growth of plants.

Table (3) Effect of different treatments on the content of phosphorous and potassium in leaves of cucumber plants treated with powdery mildew fungus

% elements		treatments
K	P	
1.18	0.248	Control
5.21	0.592	Dazim
3.47	0.364	*Tv*
3.66	0.376	*Pc*
3.36	0.398	*Pf*
4.11	0.433	*Tv+Pc*
4.19	0.450	*Tv+Pf*
4.17	0.467	*Pc+Pf*
4.98	0.528	*Tv+Pc+Pf*
1.16	0.098	L.S.D 0.05

* Each number in the table represents an average of three replicates

The effect of different treatments on the chlorophyll content of leaves and the productivity of cucumber plants treated with powdery mildew in greenhouse conditions:

The results of the study (Table 4) indicate that the treatment of the Dazim gave the highest average, reaching 11,250 mg. 100 gm-1, followed by treatments Tv + Pc + Pf, Tv + Pf, Tv + Pc and Pc + Pf, in which the average content of leaves of total chlorophyll was 10,465, 9.648, 9.618 and 9.471 mg. 100 gm^{-1}, respectively, which was significantly excelled on the control treatment in which the average content of leaves of total chlorophyll was 4.800 mg. 100 g^{-1}As for the single treatments of the bio control agent, the average content of leaves in the treatment of the bacterial bio control Pf of total chlorophyll was 9.037 mg. 100 gm^{-1}, while the fungal bio- control Tv treatment gave an average of 8.861 mg. 100 gm^{-1} followed by the treatment of fungal bio-resistant PC at a rate of 8.801 mg. 100 gm^{-1} compared to the control treatment of 4.800 mg. 100 gm^{-1}.The presence of bio control factors plays an important role in increasing the efficiency of the photosynthesis process to increase the total area of the leaf, and then there is an increase in the production of carbon compounds that contribute to the increase in

the length of the roots after moving to it [70].Several studies indicated the ability of P. fluorescens bacteria to raise the total chlorophyll rate of plant leaves, where this bacteria promotes plant growth by inducing systemic resistance as it has the ability to colonize plant roots and produce Salicylic acid and this in turn stimulates systemic control [20]. This study was in agreement with [15] on the ability of bio control agents, P. fluorescens and T. harzianum to reduce the severity of the disease of seedlings before emergence in watermelons caused by the fungus R. solani and Fusarium solani, and this in turn contributes to the positive growth of the plant and increases the general content of chlorophyll. Several researchers mentioned the efficacy of Penicillium sp. In increasing the percentage of leaves from total chlorophyll.Where [66] referred to the efficiency of the fungus P. corylophillum in encouraging and stimulating plant growth by secreting many substances, including the compound Gliotoxin, which in turn reflects positively on the growth indicators of the plant and then increases the total content of chlorophyll. As for the productivity of plants, the results of the experiment (Table 4) showed that the treatments of Dazim and Tv + Pc + Pf gave the highest rate of production per plant, which reached 716.0 and 666.0 g / plant, respectively. While the other interaction treatments for the bio control agents Tv + Pc, Tv + Pf and Pc + Pf achieved a rate of production per plant 606.0, 604.7 and 601.7 g / plant, respectively, as for the single treatments of bio control agents, the treatment of the bacterial bio-control Pf gave the highest rate. For the production of one plant, it reached 511.7 g / plant.It was followed by the treatment of the fungal bio control Pc with a production rate of 464.0 g / plant, then the fungal bio control treatment Tv, where the production rate reached 452.3 g / plant compared to the control treatment of 116.0 g / plant.This was in agreement with [50] who indicated the role of T. viride resistance in increasing the production rate of cucumber plants in the greenhouse, and this study was in agreement with [7] who indicated the efficiency of T. viride and P. fluorescens bacteria in increasing the rate of plant production in the greenhouse. The production of a single plant compared with other treatments to which bio control agents were not added. This may be due to the important role of P. fluorescens bacteria in improving the amount of production through nitrogen fixation and stimulating and encouraging plant growth [30]. Also, these bacteria have the ability to secrete 2,4-diacetyl phloroglu cinol, which encourages and stimulates plant growth [47]. These results agreed with [35] who indicated the efficiency of the bio-resistant Penicillium sp.GP16-2 in increasing the yield of tobacco plants and enhancing systemic resistance against many plant pathogens and improving its growth. [66] indicated the ability of the fungus Corylophillum Penicillium to secrete many compounds such as sesquiterpens, neoisolonifolene, anthracenes, fatty acids, alkanes and alkaloids through which it inhibits the growth and pathogenicity of the pathogenic fungus.This bio fungi also produces many stimulating and encouraging substances for plant growth, such as the compound Gliotoxin, and this, in turn, reflects positively on raising the rate of growth and production indicators in the plant.

Table (4) Effect of different treatments on chlorophyll content of leaves and productivity of cucumber plants treated with powdery mildew in greenhouse conditions.

Plant yield (gm/plant)	Chlorophyll* (mg. 100 gm-1(	Treatments
116.0	4.800	control

716.0	11.250	Dazim
452.3	8.861	*Tv*
464.0	8.801	*Pc*
511.7	9.037	*Pf*
606.0	9.618	*Tv+Pc*
604.7	9.648	*Tv+Pf*
601.7	9.471	*Pc+Pf*
666.0	10.465	*Tv+Pc+Pf*
57.2	1.888	L.S.D 0.05

* Each number in the table represents an average of three replicates

Conclusions:

1- The application of biological control agents with P. fluorescens, T.viride and P.commune and their interactions reduced the severity of powdery mildew infection and improved growth and production indicators of cucumber plants treated with powdery mildew.

2- The ability of biological control agents P. fluorescens, T.viride and P.commune and their interactions to increase the activity of peroxidase enzyme and polyphenol oxidase enzyme and this is a positive indicator in host defenses against pathogens.

3- The bio control agents of P. fluorescens, T.viride and P.commune and their interactions have the ability to increase the availability of nutrients, especially potassium and phosphorous.

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